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A Broadly Neutralizing Human Monoclonal Antibody against gp41 of Human Immunodeficiency Virus Type 1

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ABSTRACT

We have established a hybridoma clone, designated 2F5, secreting a neutralizing human monoclonal antibody (MAb) specific for gp41 of human immunodeficiency virus type 1 (HIV-1). The epitope of MAb 2F5 was mapped to amino acid sequence Glu-Leu-Asp-Lys-Trp-Ala on the ectodomain of gp41. In this study different *in vitro* test systems were used to characterize the neutralizing properties of MAb 2F5. In syncytium inhibition assays, fusion inhibition experiments, and neutralization assays on different HIV-susceptible cells (H9, U937, and peripheral blood mononuclear cells) MAb 2F5 showed broad-spectrum neutralizing capacity against HIV-1 laboratory isolates IIIB, MN, RF, and SF2. In addition, primary isolates from AIDS patients were also neutralized.

INTRODUCTION

THE IMMUNE RESPONSE developing subsequent to infection with HIV-1 consists of both humoral and cellular mechanisms which may be important for the control of viral replication.¹⁻³ A humoral response to all HIV-1 protein antigens has been observed. However, the envelope glycoprotein, which consists of the outer membrane gp120 and transmembrane gp41, elicits most of the neutralizing activity found in human sera. The induction of broadly reactive neutralizing antibodies is complicated by the presence of several highly variable regions on the envelope glycoprotein. Antibodies against the principal neutralizing determinant (PND) on the V3 loop of gp120 predominantly provide type-specific neutralization⁴⁻⁶ and only in some cases has cross-reactivity been reported.⁷ There are, however, several conserved structures that are important targets for effective virus neutralization. Antibodies recognizing the CD4-binding domain on gp120 can have broadly reactive neutralizing activity. This domain is a large, conformation-dependent discontinuous epitope on the surface of the gp120 molecule.⁸⁻¹¹ Portions of the second, third, and fourth conserved regions of gp120 are involved in CD4 binding. Antibodies to these regions are able to block infection of the majority of HIV-1 strains by interfering with binding to CD4-positive cells.^{10,12}

The neutralizing activity of sera from HIV-1-seropositive individuals is not solely mediated by anti-gp120 antibodies, but also by anti-gp41 antibodies. This has been demonstrated by Broliden and co-workers,¹³ who showed that seroreactivity to several gp41-derived peptides correlates with partial cross-neutralization activity. Antibodies against gp41 are mainly directed against the immunodominant region near the N terminus of gp41. However, antibodies to this region are not neutralizing. Monoclonal antibodies against gp41 recognizing epitopes outside this immunodominant region are not frequently described and show no^{14,15} or only weak¹⁶ neutralizing capacities (for review, see Ref. 17).

Several *in vitro* biological assays have been developed that are rapid, sensitive, and make it possible to measure the specificity and relative potency of neutralizing antibodies on a number of target cells with a broad spectrum of HIV variants, including primary isolates.

Different parameters are measured as an indication of virus replication and are used as assay end points. Syncytia or plaque formation^{18,19} and measuring of viral products such as reverse transcriptase²⁰ or p24 antigen²¹ are considered reliable parameters to monitor virus production.

We have previously established a panel of 28 human monoclonal antibodies against gp41 and gp120.²² Of this panel, seven monoclonal antibodies (MAbs) showed neutralizing ac-

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tivity against HIV-1. The broadly reactive neutralizing properties of an MAbs that is directed against gp41 and that recognizes the amino acid sequence Glu-Leu-Asp-Lys-Trp-Ala (ELDKWA) are described in this study.

MATERIALS AND METHODS

Monoclonal antibody 2F5

Production and characterization of human monoclonal antibody 2F5 have been previously described in detail.²² Hybridomas were generated by a combined polyethylene glycol/electrofusion method.²³ Briefly, peripheral blood mononuclear cells (PBMCs) from naturally infected asymptomatic HIV-1-seropositive blood donors were used as HIV-1-sensitized cells. Lymphocytes were mixed with the heteromyeloma cell line CB-F7 in a ratio of 5:1 in medium containing 5% polyethylene glycol and were pulsed in plastic cuvettes (Bio-Rad, Richmond, CA). Hybridoma supernatants were screened for HIV-specific IgG production. Further characterization of the HIV-specific IgG was done by enzyme-linked immunosorbent assay (ELISA), using recombinant HIV-1 proteins, Western blotting, and immunofluorescence assays. Monoclonal antibody 2F5 belongs to subclass IgG₃²² and has been demonstrated to recognize amino acid sequence ELDKWA on the ectodomain of gp41.²⁴

Viruses and cells

All HIV-1-infected cell lines were grown in cell culture medium (CCM): RPMI 1640 medium (Biochrom, Berlin, Germany) containing 10% heat-inactivated fetal calf serum (FCS), 100 U of penicillin, 100 µg of streptomycin, and 2 µg of Polybrene (Sigma, St. Louis, MO) per milliliter. Noninfected cell lines were cultivated in RPMI 1640 medium with 10% heat-inactivated FCS, in the absence of antibiotics and Polybrene. Peripheral blood mononuclear cells from healthy HIV-seronegative donors were grown in RPMI 1640 with 10% heat-inactivated FCS, supplemented with interleukin 2 (IL-2; Biotest, Dreieich, Germany) in the absence of antibiotics. When PBMCs were used for virus titration, coculture, or neutralization assays, antibiotics and Polybrene were added to the medium.

H9 cells were obtained from R. Gallo (Laboratory of Tumor Cell Biology, Bethesda, MD). The cell lines H9/HTLVIII_B, U937, and Molt-4 were obtained from the American Type Culture Collection (ATCC, Rockville, MD).

The following reagents were obtained through the AIDS Research and Reference Reagent Program, Division of AIDS, National Institute of Allergy and Infectious Diseases (NIAID, Bethesda, MD): cell line AA-2 from M. Herschfield,²⁵ HUT78/HIV-1_{SF2} from J. Levy,^{26,27} and H9/HTLV-III_{MN} NIH 1984^{28,29} and H9/HTLVIII_{RF} NIH-1983^{30,31} from R. Gallo.

Virus stocks of HIV-1 isolates IIIB, MN, RF, and SF2 were produced in H9 cells. Two to 3 weeks after infection, cell-free supernatants were harvested and frozen in aliquots at -80°C. Isolate R640 was obtained by passaging isolate HTLVIII_B on B cells from a healthy HIV-1-seronegative blood donor. Cell-free supernatant from these B cells was harvested and frozen in aliquots at -80°C. Isolate R548 was derived from isolate HIV-

1_{WR-SK/86}.³² Cell-free supernatant from HIV-1_{WR-SK/86}-infected macrophages was used to infect primary B cells from an HIV-1-seronegative donor. The virus has been passaged for 6 months in B cells. Cell-free supernatant was harvested and frozen in aliquots at -80°C.

Primary isolates WYG and WRF were obtained from two different AIDS patients. HIV-1-infected PBMCs were isolated from heparinized venous blood by a Ficoll (Biochrom) density gradient centrifugation and were co-cultivated with 2×10^6 phytohemagglutinin (PHA)-stimulated HIV-1 negative donor PBMCs per milliliter. Cultures were subsequently monitored for the presence of p24 antigen in the supernatants. Phytohemagglutinin-stimulated donor PBMCs were added weekly. After 3 to 4 weeks cell-free supernatants from WYG- and WRF-infected PBMCs were harvested and frozen in aliquots at -80°C.

Determination of p24

p24 was determined using a commercially available ELISA kit (HIV-1 p24 antigen capture assay; Coulter Corp., Hialeah, FL) as well as an in-house p24 antigen assay. Samples for both assays were treated with Nonidet P-40 (NP-40; U.S. Biochemical, Cleveland, OH) at a final concentration of 2% and incubated for 1 hr to inactivate virus. The Coulter HIV-1 p24 antigen capture assay was performed according to manufacturer instructions. The detection limit of this assay was approximately 10 pg/ml.

The in-house p24 antigen assay was performed as a double-antibody sandwich ELISA. A murine monoclonal antibody against p24 was bound to a Maxisorp ELISA plate (Nunc, Roskilde, Denmark) at a concentration of 2 µg/ml in 0.1 M sodium carbonate buffer, pH 9.5. Samples were incubated for 1 hr at room temperature. Captured p24 antigen was complexed with a horseradish peroxidase-labeled human monoclonal antibody against p24. The complex was detected by incubation with 1,2-*o*-phenylenediamine dihydrochloride solution containing 0.04% H₂O₂. The reaction was stopped with 1.25 M sulfuric acid. The optical density (OD) was read at 492 nm using an automated plate reader (EAR 400AT; SLT, Grödig, Austria). The reference wavelength was 620 nm. The absorbance of each well was calibrated against the absorbance of a p24 standard curve. The detection limit of the assay is approximately 40 pg/ml.

Immunofluorescence assay

HIV-1-infected cells and noninfected cells were washed twice with PBS, resuspended in PBS at 5×10^6 cells/ml, and 10 µl of the cell suspension was dropped on different reaction fields of adhesion slides (Bio-Rad) according to manufacturer instructions. After sedimentation of the cells, slides were fixed with acetone (10 min at 4°C). The following incubation steps were done in a wet chamber. Cells were blocked with heat-inactivated goat serum diluted 1:5 in phosphate-buffered saline (PBS) for 30 min (20 µl/reaction field) followed by incubation of 20 µl of antiserum or antibody dilution (5–10 µg/ml) for 1 hr at room temperature. Slides were then washed three times for 5 min with PBS before incubation with fluorescein isothiocyanate conjugated to goat anti-human IgG (Sigma Chemicals). After incubation for 30 min slides were washed three times and mounted with 50% glycerol in PBS.

Syncytium inhibition assay/cell-free virus inoculum

The assays were carried out with HIV-1 strains IIIB, MN, RF, SF2, R640, R548, WYG, and WRF. The stocks were titrated on AA-2 cells using eight replicates per fivefold dilution step and the 50% tissue culture infective dose (TCID₅₀) per milliliter was calculated by the method of Reed and Muench.³³ Virus stocks were diluted to 10²–10³ TCID₅₀/ml in CCM and used as virus inoculum.

As a positive control, a serum pool from HIV-1-seropositive symptomatic patients (anti-HIV) was used. Sera from healthy HIV-1-seronegative donors were used as negative controls. The assay was carried out as follows.

Fifty microliters of the appropriate virus dilution and 50 μ l of MAb, anti-HIV, or control samples (12 serial 2-fold dilutions) were preincubated for 2 hr at 4°C. The MAb concentration in the lowest dilution was 100 μ g/ml and the lowest anti-HIV dilution used was 1:40. The cells were infected by adding the preincubated virus-antibody mixture to 100 μ l of AA-2 cell suspension (5 \times 10⁵ cells/ml). The cells were then cultivated for 5 days at 37°C, 5% CO₂ followed by microscopic evaluation of syncytium formation. Occurrence of at least one syncytium per well was recorded as an indication of HIV-1 infection. All experiments were done in 96-well plates (Nunc) with eight replicates per dilution step. The 50% effective concentration (EC₅₀) was calculated by the method of Reed and Muench.³³ This is defined as the antibody concentration producing a 50% reduction in the number of wells in which syncytia were observed. Calculated concentrations correspond to a volume of 100 μ l present at the preincubation step (virus plus antibody). All syncytium inhibition experiments included a titration of the virus inoculum to confirm the infectious titer actually used. In addition, plots of antibody concentration versus syncytium inhibition using 96 replicates per dilution step under conditions identical to those described above were also performed.

Fusion inhibition assay

The fusion inhibition assay was carried out as described for the syncytium inhibition assay with the following modifications: instead of cell-free culture supernatant, H9 or Molt-4 cells infected with HIV-1 isolates IIIB, MN, RF, SF2, R640, and R548 were used as virus inoculum. The chronically infected cells were obtained by infecting 2 \times 10⁶ cells with 1 ml of cell-free supernatant of isolates IIIB, MN, RF, SF2, R640, and R548. Cells were allowed to undergo the destructive growth phase. The virus-infected cells that emerged following the initial crisis were passaged. After approximately 3 to 4 weeks, chronically infected cells (infection rate, 70–95%; determined by immunofluorescence assay) were washed twice with CCM and diluted to 1–2 \times 10³ cells/ml in CCM. Fifty microliters of these cells suspensions was preincubated with 50 μ l of serial twofold dilutions of antibody and anti-HIV for 2 hr at 4°C. Indicator cells (100 μ l of AA-2 cell suspension; 5 \times 10⁵/ml) were then added and cultivated at 37°C, 5% CO₂. Syncytium formation was inspected by light microscopy after 24–48 hr and the EC₅₀ values were evaluated as described above.

Neutralization assay

Neutralization assays were performed on different HIV-susceptible cells (H9, U937, and PHA-stimulated PBMCs from

healthy HIV-seronegative blood donors) using p24 as virus replication marker. For the assays on the cell lines H9 and U937 HIV-1 isolates IIIB, MN, and RF were used. The laboratory isolates IIIB and MN as well as the two primary isolates WYG and WRF were used in the assays with PBMCs. Fifty microliters of HIV (10²–10³ TCID₅₀/ml, which was determined on the respective cells) was preincubated with serial dilutions (10 twofold steps starting at a concentration of 50 μ g/ml) of MAbs for 2 hr at 4°C, and then the virus-antibody mixture was added to 100 μ l of HIV-susceptible cells (H9 or U937 cells at a cell density of 1 \times 10⁶ cells/ml or PBMCs at 4 \times 10⁶ cells/ml). Assays were done in 96-well plates with 8 replicates per antibody dilution step. A titration of the virus inoculum was included in each experiment. Fivefold dilutions of the virus inoculum were made and 50 μ l of each virus dilution was mixed with 50 μ l of CCM and 100 μ l of cell suspension. The cutoff value for the p24 assay was established by addition of 50 μ l of virus inoculum with 150 μ l of CCM to each of eight wells in each test plate. The cutoff level for p24 positivity was chosen to be values greater than 2 SD above the mean value for the eight wells containing the virus input inoculum.

Neutralization assays with H9 cells were incubated for 7 days, and the plates with U937 and PBMCs were cultivated for 2 weeks with the addition of 50 μ l of fresh medium on day 6 postinfection (p.i.). After this incubation period 50 μ l of the supernatant of each well was collected, diluted 1:5 in CCM containing NP-40 (final concentration, 2%), and the p24 antigen assay was performed qualitatively for each well. The presence of p24 antigen in the supernatant was used as a parameter for virus replication. Each well was characterized qualitatively as infected or noninfected and the EC₅₀ was calculated by the method of Reed and Muench.³³

Following determination of the neutralization titer as described above, neutralization was also assessed by plotting V_p/V_0 , the ratio of the amount of p24 in the presence of MAb to the amount of p24 in its absence, against the MAb concentration. This was carried out by pooling all eight wells for each antibody dilution step and determining the p24 antigen quantitatively.

RESULTS

Syncytium inhibition assays

The cell-free HIV-1 laboratory strains IIIB, MN, RF, and SF2 and the B cell-propagated viruses R640 and R548 were titrated on AA-2 cells to check their capability to infect AA-2 cells and to determine the infectious titers of the virus stocks. They were diluted to 10²–10³ TCID₅₀/ml with CCM and used as virus inocula. Antibody 2F5 inhibited syncytium formation with all isolates tested (Table 1). The 50% effective concentrations (EC₅₀) ranged between 0.7 μ g/ml for the MN strain and 9.7 μ g/ml for RF. The primary isolates WYG and WRF also infected AA-2 cells and induced syncytium formation. Monoclonal antibody 2F5 inhibited syncytium formation of primary isolates WYG and WRF at EC₅₀ values of 5.3 and 5.7 μ g/ml, respectively.

To allow a more exact determination of the syncytium inhibition characteristics of antibody 2F5, syncytium inhibition ex-

TABLE 1. SYNCYTIIUM-INHIBITING CAPACITY OF MONOCLONAL ANTIBODY 2F5 AND A NEUTRALIZING PATIENT SERUM POOL (ANTI-HIV)^a

HIV-1 laboratory isolates	EC ₅₀	
	MAb 2F5 (μg/ml)	Anti-HIV serum dilution
IIIB	3.7	1:830
MN	0.7	1:5120
RF	9.7	1:960
SF2	0.8	1:830
R640	1.1	ND
R548	4.8	ND
HIV-1 primary isolates		
WYG	5.3	1:208
WRF	5.7	1:310

^aSyncytium inhibition assays were performed on AA-2 cells by using cell-free virus inoculum. Data are expressed as the mean 50% effective concentrations (in μg/ml) for MAb 2F5 and as serum dilution for anti-HIV, calculated by the method of Reed and Muench,³³ and are derived from at least three independent experiments.

ND, not determined.

periments were done with 96 replicates per antibody dilution step using cell-free HIV-1 isolates IIIB, MN, RF, and SF2. Figure 1 shows the syncytium inhibition curves of representative experiments. It confirms the data from experiments presented in Table 1 in that strain MN was most sensitive and RF least sensitive to neutralization by MAb 2F5. It also demonstrated that 100% syncytium inhibition of all four strains tested could be obtained with approximately 50 μg of antibody per milliliter.

Fusion inhibition assays

In fusion inhibition assays using cell-associated virus, MAb 2F5 could block fusion between H9 or Molt-4 cells chronically

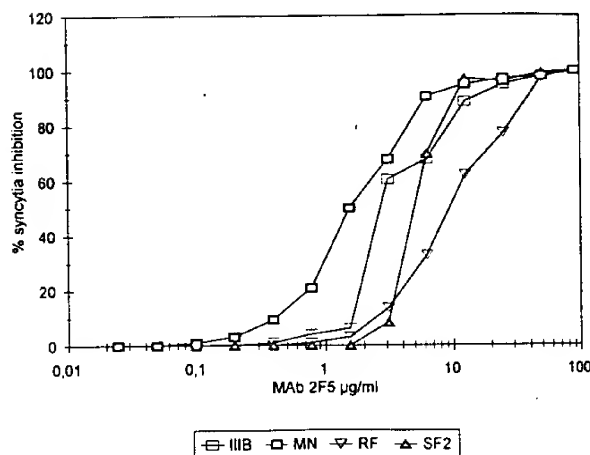


FIG. 1. Syncytium inhibition characteristics of antibody 2F5. Cell-free HIV-1 isolates IIIB, MN, RF, and SF2 were used as virus inocula in syncytium inhibition assays. Studies were done with 96 replicates per antibody dilution as described in Materials and Methods. Results of one representative study are shown.

infected with HIV-1 IIIB, MN, RF, SF2, R640, or R548 and the indicator cell line AA-2. The EC₅₀ concentrations (in μg/ml) of MAb 2F5 are shown in Table 2. Again, as shown in the syncytium inhibition assays, these results revealed that isolates MN and SF2 are most sensitive and isolate RF is least sensitive to neutralization by MAb 2F5. The 50% effective concentrations in these assays range from 3.4 μg/ml for Molt-4/SF2 to 29.8 μg/ml for Molt-4/RF cell-associated virus and 17.7 μg/ml for H9/SF2 to 27.3 μg/ml for H9/R640. However, fusion with H9/RF could not be inhibited at the concentrations tested (i.e., up to 50 μg/ml). Compared to the EC₅₀ values obtained in the syncytium inhibition assays using cell-free virus inoculum approximately 20 times higher antibody concentrations were necessary to inhibit fusion between the indicator cells AA-2 and chronically infected H9 cells and 3 to 6 times higher antibody

TABLE 2. FUSION INHIBITION CAPACITY OF MONOCLONAL ANTIBODY 2F5 AND NEUTRALIZING PATIENT SERUM ANTI-HIV^a

HIV-1 isolate	EC ₅₀			
	MAb 2F5 (μg/ml)		Anti-HIV dilution	
	H9/inf	Molt-4/inf	H9/inf	Molt-4/inf
IIIB	21.0	13.6	<1:80	1:120
MN	19.3	4.4	1:160	1:190
RF	>50	29.8	<1:80	<1:80
SF2	17.7	3.4	<1:80	1:350
R640	27.3	ND	ND	ND
R548	ND	17.7	ND	ND

^aFusion between cell-associated virus (chronically infected H9 or Molt-4 cells) and AA-2 cells was monitored. Data are expressed as EC₅₀ (in μg/ml) for MAb 2F5 and serum dilution for anti-HIV, calculated by the method of Reed and Muench.³³

ND, not determined.

concentrations were necessary to inhibit fusion between AA-2 cells and chronically infected Molt-4 cells.

Neutralization assays

The broad syncytium-inhibiting and fusion-inhibiting potency of MAb 2F5 was confirmed by neutralization assays using the T cell line H9 and the monocytoid cell line U937. p24 production was used as an indicator of virus replication and the 50% neutralizing concentrations (EC_{50}) were calculated by the method of Reed and Muench.³³ The EC_{50} values obtained for HIV-1 strains IIIB, MN, RF, and SF2 were comparable to the results obtained by syncytium inhibition assays using AA-2 cells (Table 3). Improved neutralization capacity against HIV-1 RF was observed with the U937 cell line, for which the EC_{50} was 0.1 $\mu\text{g/ml}$ compared to 10.9 $\mu\text{g/ml}$ for the neutralization assay with H9 cells (Table 3) and 9.7 $\mu\text{g/ml}$ for the syncytium inhibition assay with AA-2 cells (Table 1). Monoclonal antibody 2F5 was also tested against the IIIB and MN strains as well as against the primary isolates WYG and WRF in neutralization assays using PBMCs as HIV-1-susceptible cells. The 50% neutralizing concentrations calculated were 0.8 $\mu\text{g/ml}$ for IIIB, 0.1 $\mu\text{g/ml}$ for MN, 10.3 $\mu\text{g/ml}$ for isolate WYG, and 5.4 $\mu\text{g/ml}$ for isolate WRF.

In addition to the calculated EC_{50} we measured the amount of p24 antigen in the cultures containing MAb (V_n) and in the control cultures without MAb (V_0). The ratio V_n/V_0 was plotted versus the MAb concentration to allow an estimation of 90% neutralizing concentrations. Figure 2 shows the results of representative neutralization assays using HIV-1 isolates IIIB, MN, and RF on H9 cells. The amounts of p24 in the control cultures without MAb on day 7 were 618, 77, and 81 ng of p24 per milliliter of supernatant in the IIIB-, MN-, and RF-infected cultures, respectively. Ninety percent reduction of p24 could be achieved with 0.4, 0.08, and 1.8 μg of MAb 2F5 per milliliter for the isolates IIIB, MN, and RF, respectively.

The neutralizing capacity of MAb 2F5 against the primary isolate WYG in a neutralization assay on PBMCs is shown in

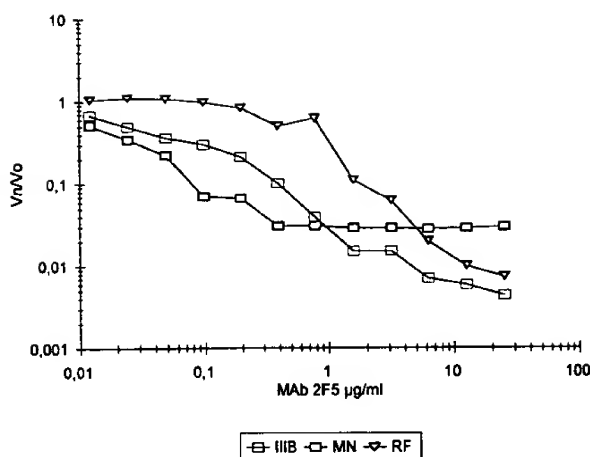


FIG. 2. Neutralization capacity of human MAb 2F5 against laboratory isolates IIIB, MN, and RF. Assays were performed on H9 cells using p24 production as replication marker as described in Materials and Methods. The amount of p24 in the cultures without MAb was 618, 77, and 81 ng/ml for IIIB, MN, and RF, respectively.

Fig. 3. The 90% neutralizing concentration was approximately 4.5 $\mu\text{g/ml}$ and the amount of p24 in the control culture without MAb was 182 ng/ml.

DISCUSSION

Significant obstacles to the creation of an effective AIDS vaccine are the wide diversity between HIV-1 isolates from North America, Europe, and Africa³⁴ as well as the high antigenic variability that can occur in viruses isolated from a single HIV-1-infected individual during the course of the infection.^{35,36} To overcome or control HIV-1 infection it will be necessary to define highly conserved regions of HIV-1 that can in-

TABLE 3. NEUTRALIZING ACTIVITY OF MONOCLONAL ANTIBODY 2F5 AGAINST DIFFERENT HIV-1 ISOLATES, USING CELL-FREE VIRUS AS INOCULUM

HIV isolates	EC_{50} of MAb 2F5 ($\mu\text{g/ml}$)		
	Assay system/test cells		
	H9	U937	PBMC
IIIB	0.3	0.13	0.8
MN	0.5	0.3	0.1
RF	10.9	0.1	ND
SF2	ND	0.1	ND
WYG	ND	ND	10.3
WRF	ND	ND	5.4

*Neutralization assays were performed on cell lines H9 and U937 and on PBMCs. Data are expressed as EC_{50} (in $\mu\text{g/ml}$) of MAb 2F5 and are calculated by the method of Reed and Muench.³³

ND, not determined.

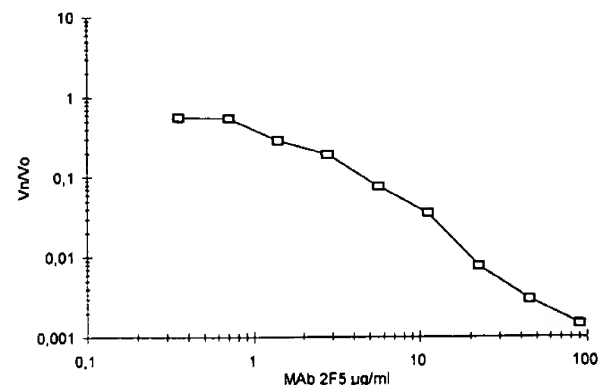


FIG. 3. Neutralization capacity of human MAb 2F5 against primary isolate WYG. The assay was performed on PBMCs, using p24 production as replication marker as described in Materials and Methods. The amount of p24 in the culture without MAb was 182 ng/ml.

duce protective immune responses and that can elicit broadly neutralizing antibodies.

The transmembrane glycoprotein gp41 of HIV-1 is relatively conserved.³⁷ Hypervariable regions as commonly seen in gp120 are not observed in gp41. The antibody described in this study (MAb 2F5) was found to recognize the transmembrane glycoprotein gp41 outside the immunodominant region. The epitope of this antibody was mapped to the amino acid sequence ELDKWA, corresponding to amino acids 662–667 of gp160 on HIV-1 isolate BH10, and this sequence has been found in 72% of current HIV-1 isolates described.²⁴ The high conservation of this gp41 epitope is also reflected in our data, in which the anti-gp41 antibody 2F5 shows a broad neutralization capacity. Antibody 2F5 neutralized laboratory strains IIIB, MN, RF, SF2, R640, and R548 in a group-specific manner. The EC₅₀ ranged from 0.7 to 9.7 µg/ml, when cell-free virus inocula were tested in syncytium inhibition assays on AA-2 cells. Moreover, antibody 2F5 neutralized primary isolates obtained from HIV-1-infected individuals. In this study we also describe two primary isolates, which were neutralized at EC₅₀ values of approximately 5 µg/ml. Significantly higher amounts of antibody were necessary to block cell fusion via chronically infected H9 or Molt-4 cells (see Table 2). Compared to the EC₅₀ values obtained against cell-free virus, significantly higher concentrations of antibody were necessary to inhibit fusion between the indicator cells AA-2 and chronically infected H9 cells and Molt-4 cells. Similar results were obtained when a neutralizing serum pool from HIV-1-positive individuals (anti-HIV) was tested in fusion inhibition assays (Tables 1 and 2), which is in accordance with the observations of Langlois *et al.*³⁸ and Rusche *et al.*⁴ This indicates that these observations are not a particular phenomenon associated with antibody 2F5, but probably reflect the higher levels of expression of gp120/41 complexes on the surfaces of the infected cells. With the exception that cell fusion by H9 cells infected with isolate RF could not be prevented with 50 µg of antibody 2F5 per milliliter, the results of the fusion inhibition assays again demonstrated the group-specific neutralization capacity of antibody 2F5. We also examined the neutralizing activity of MAb 2F5 in different neutralization assay systems, using the cell lines H9 and U937 as well as PBMCs as HIV-1-susceptible cells. As shown in Table 3 and Figs. 2 and 3, MAb 2F5 could neutralize the virus isolates tested in the different assay systems.

A comparison of the graphically estimated 90% neutralizing concentrations and the calculated 50% effective concentrations indicated that both methods resulted in similar neutralizing concentrations. The neutralization curves shown in Figs. 2 and 3 allow an interpretation of the neutralization dependence of the MAb concentrations whereas the method of Reed and Muench,³³ by which only 50% end points are calculated, resulted in a more stringent estimation of the neutralizing capacity of MAb 2F5.

These data emphasize the efficacy of a monoclonal antibody generated against a conserved envelope region in neutralizing widely divergent laboratory strains and isolates from infected persons. MAb 2F5 has been characterized in a similar way in another study³⁹ and showed broadly neutralizing potency. Monoclonal antibody 2F5 has also been demonstrated to be one of the most potent MAbs with respect to neutralization of HIV-

1 isolates IIIB and MN in an NIAID international collaborative study.⁴⁰

Although most of the MAbs directed against the PND region of gp120 show a type-specific neutralization pattern,^{4,5,41} a few anti-V3 antibodies mediating broad neutralizing activity have been described^{7,21} that are more broadly reactive than antibodies directed against the CD4-binding domain on gp120.

The low frequency of emergence of neutralization-resistant virus variants in the presence of MAb 2F5 (M. Purtscher, A. Trkola, and G. Gruber, unpublished observations) and the high level of conservation of the 2F5 epitope among HIV-1 strains suggest that this envelope region may play an important role in the viral life cycle and suggest that neutralizing antibodies generated against this epitope might be effective in the immunotherapy of AIDS or as a passive immunization.

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